

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

**The rediscovery of uromodulin (Tamm-Horsfall protein): from tubulointerstitial nephropathy to chronic kidney disease**

**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/89120> since 2016-01-07T19:04:49Z

*Published version:*

DOI:10.1038/ki.2011.134

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



# UNIVERSITÀ DEGLI STUDI DI TORINO

***This is an author version of the contribution published on:***

*Questa è la versione dell'autore dell'opera:*

*Rampoldi L, Scolari F, Amoroso A, Ghiggeri G, Devuyst O. The rediscovery of uromodulin (Tamm-Horsfall protein): from tubulointerstitial nephropathy to chronic kidney disease. Kidney Int. 2011 Aug;80(4):338-47; doi: 10.1038/ki.2011*

***The definitive version is available at:***

*La versione definitiva è disponibile alla URL:*

*[<http://www.nature.com/ki/journal/v80/n4/full/ki2011134a.html>]*



**The rediscovery of uromodulin (Tamm-Horsfall protein):  
from tubulo-interstitial nephropathy to chronic kidney  
disease**

Journal:	<i>Kidney International</i>
Manuscript ID:	KI-01-11-0050.R1
Manuscript Type:	Minireview
Date Submitted by the Author:	02-Mar-2011
Complete List of Authors:	Rampoldi, Luca; Dulbecco Telethon Institute c/o San Raffaele Scientific Institute, Division of Genetics and Cell Biology Scolari, Francesco; Hospital of Montichiari, Division of Nephrology Amoroso, Antonio; University of Turin, Department of Genetics, Biology and Biochemistry Ghiggeri, Gian Marco; G. Gaslini Institute, Nephrology, Dialysis, Transplant Devuyst, Olivier; University of Zurich, Institute of Physiology
Subject Area:	Cell Biology , Genetics
Keywords:	chronic kidney disease, genetic renal disease, interstitial fibrosis, cystic kidney, malfolding proteins

SCHOLARONE™  
Manuscripts

**The rediscovery of uromodulin (Tamm-Horsfall protein): from tubulo-interstitial  
nephropathy to chronic kidney disease**

<sup>1</sup>Luca Rampoldi, Ph.D.\*, <sup>2</sup>Francesco Scolari, M.D., <sup>3</sup>Antonio Amoroso, M.D., <sup>4</sup>GianMarco  
Ghiggeri, M.D. and <sup>5</sup>Olivier Devuyst, M.D., Ph.D.

<sup>1</sup>Dulbecco Telethon Institute c/o Division of Genetics and Cell Biology, San Raffaele Scientific  
Institute, Milan, Italy; <sup>2</sup>Division of Nephrology, Montichiari Hospital, University of Brescia,  
Brescia, Italy; <sup>3</sup>Department of Genetics, Biology and Biochemistry, University of Turin, Turin, Italy;  
<sup>4</sup>Division of Nephrology, G. Gaslini Institute, Genoa, Italy; <sup>5</sup>Institute of Physiology, University of  
Zurich, Zurich, Switzerland

\*Correspondence to: Luca Rampoldi Luca, Dulbecco Telethon Institute c/o Molecular Genetics of  
Renal Disorders Unit, Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Via  
Olgettina 58, 20132 Milan, Italy. Phone +39 0226434947; fax +39 0226434767; email  
[rampoldi.luca@hsr.it](mailto:rampoldi.luca@hsr.it)

Financial support:

This work was supported by Telethon-Italy [TCR08006 to L.R.]; the European Community's 7<sup>th</sup>  
Framework Program [HEALTH-F2-2007-201590, EUNEFRON program, to O.D. and L.R.];  
Belgian agencies FNRS and FRSM [to O.D.]; the 'Fondation Alphonse & Jean Forton' [to O.D.]; a  
Concerted Research Action [05/10-328 to O.D.]; an Inter-university Attraction Pole [IUAP P6/05 to  
O.D.]; the DIANE project (Communauté Française de Belgique) [to O.D.] and the National Centre

of Competence in Research (NCCR) “Kidney.CH” [to O.D.]. L.R. is an Associate Telethon Scientist.

Running Title:

Uromodulin and chronic diseases of the kidney

Word count:

4837

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**ABSTRACT**

Uromodulin (Tamm-Horsfall protein) is the most abundant protein excreted in the urine under physiological conditions. It is exclusively produced in the kidney and secreted into the urine via proteolytic cleavage. Its biological function is still not fully understood. Uromodulin has been linked to water/electrolyte balance and to kidney innate immunity. Also, studies in knock-out mice demonstrated that it has a protective role against urinary tract infections and renal stone formation. Mutations in the gene encoding uromodulin lead to rare autosomal dominant diseases collectively referred to as uromodulin-associated kidney diseases. They are characterised by progressive tubulo-interstitial damage, impaired urinary concentrating ability, hyperuricaemia, renal cysts and progressive renal failure. Novel *in vivo* studies point at intracellular accumulation of mutant uromodulin as a key primary event in the disease pathogenesis. Recently, genome-wide association studies identified uromodulin as a risk factor for chronic kidney disease and hypertension and suggested that the level of uromodulin in the urine could represent a useful biomarker for the development of chronic kidney disease. In this review, we summarise these recent investigations, ranging from invalidation studies in mouse to Mendelian disorders and to genome-wide association, which led to a rediscovery of uromodulin and boosted the scientific and clinical interest for this ancient molecule.

**KEYWORDS**

Uromodulin, Tamm-Horsfall protein, Chronic kidney disease

## Biology of uromodulin

In the early fifties, Igor Tamm and Frank Horsfall described a mucoprotein that could be purified from urine and inhibited viral haemagglutination (1). The protein, referred to as Tamm-Horsfall protein (THP), is the most abundant protein in urine under physiological conditions (2). In 1985, Muchmore and Decker isolated a protein from the urine of pregnant women that they called uromodulin in relation with its immunosuppressive activity documented *in vitro* (3). Two years later, THP and uromodulin were shown to be the same protein in a seminal work by Pennica et al. (4). The term uromodulin will be used hereafter in this review.

### *Protein sequence and domain composition*

Uromodulin is synthesised as a 640 amino acids precursor. The protein enters the secretory pathway where it is GPI-anchored, glycosylated and sorted to the apical plasma membrane of epithelial cells. The rate-limiting step in uromodulin maturation is the processing in the endoplasmic reticulum (ER), likely because of the complex tertiary structure given by its high number of cysteine residues (48; 7% of amino acid content) all engaged in the formation of intra-molecular disulphide bonds (5). The molecular weight of uromodulin (105 kDa) is significantly contributed (30%) by N-glycosylation. Evidence for O-glycosylation has also been reported (6). The presence of such high glycan content is important for the chemico-physical properties and function of uromodulin (see below). The domain composition of uromodulin includes a leader peptide directing its entry in the secretory pathway, three EGF-like domains (EGF-II and EGF-III predicted to be calcium-binding), a central domain of unknown function (named D8C as it contains 8 conserved cysteines), a zona pellucida (ZP) domain and a glycosylphosphatidylinositol (GPI)-anchoring site (predicted at position 614) (Figure 1a). EGF-like domains are found in many secreted and extracellular proteins and are thought to play a role in processes as adhesion, coagulation, and receptor–ligand interaction.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

In uromodulin, these modules are likely important for protein-protein interaction. The ZP domain is found in a variety of extracellular eukaryotic proteins, e.g. sperm receptors ZP1 and ZP3, tectorial membrane components alpha- and beta-tectorin, and is essential for protein polymerisation (7) (Figure 1b). Indeed, uromodulin is mainly present in the urine as a high molecular weight polymer ( $M_r$  1-10x10<sup>6</sup> daltons) that appears at the electron microscopy analysis as a matrix composed by fibrils with a width of about 100 Å and an average length of 25,000 Å. Depending on the ionic conditions uromodulin matrices can form a gel-like structure that is water impermeable but allows ion movement (8).

*Timing and tissue-specificity of expression*

Uromodulin is a kidney-specific protein that is exclusively expressed by epithelial cells lining the thick ascending limb (TAL) of Henle’s loop (9). It is mainly located at the apical plasma membrane (10) although localisation at the basolateral side of TAL cells has also been reported (11). A basolateral release of uromodulin is also suggested by studies on its trafficking in transfected polarised epithelial cells and by its presence at very low concentrations in the blood (12). The presence of uromodulin protein and transcript is detected from embryonic day 16.5 in the developing mouse kidney (13, 14). In humans, the protein was detected from gestational week 16 in immunohistochemistry analysis and from week 20 in the amniotic fluid (15). Its expression steadily increases with time and maturation of TAL tubules till after birth. Uromodulin is produced at a very high rate by mature TAL cells being their most abundant transcript (16). The half-life of the protein is rather short (about 9 hours in rabbit and 16 hours in humans) (17), due to its high rate of secretion in the urine that ranges from 20 to 100 mg/day in humans under physiological conditions (18). Uromodulin is released from the apical plasma membrane of epithelial cells into the tubule lumen via a conserved proteolytic cleavage (19). Cleavage is necessary for protein polymerisation, as it releases an inhibitory motif that prevents premature protein assembly (20), similarly to what



described for zona pellucida protein ZP3 (21) (Figure 1b). Interestingly, data from our studies in transfected MDCK cells (20) and from urine peptidomes (22) suggest the presence of an alternative cleavage distal to the inhibitory motif releasing monomeric uromodulin. Little information is currently available on the presence of a specific protease(s) involved in uromodulin excretion in the urine and on how this is regulated.

### *Evolutionary conservation*

The sequence and domain composition of uromodulin is very similar to the one of glycoprotein 2 (GP2), that is the major component of zymogen granule membranes of exocrine pancreas, and liver-specific ZP domain-containing protein (LZP). *GP2* and *UMOD* genes lie adjacent on chromosome 16p12.3 suggesting that they could have evolved through duplication-divergence of a common ancestral gene. As uromodulin (see below), GP2 protein is able to bind to *E. coli* of the fimbriated type I suggesting that the two proteins exert similar protective functions in the urinary and digestive systems (23).

Uromodulin is present in the kidney of all mammals. Immunoreactive protein in the layers of the skin of several amphibians and fishes, and in the distal tubules of the kidney of some amphibians was reported (24). Comparative genomics analysis reveals putative *UMOD* homologues in amphibian (*X. tropicalis*) and fish (*D. rerio*) genomes with significant sequence similarity at the predicted protein level. The function of these homologues and their relevance for comparative physiology remain to be determined.

### *Biological function*

The biological function of uromodulin is still rather elusive. Uromodulin has been hypothesised to have a role in water/electrolyte balance in the TAL. This hypothesis is based on its gelification and physico-chemical properties (8) and on the evidence that its expression is increased by a high-salt

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

diet and by chronic administration of the loop diuretic furosemide (25). More direct evidence comes from a recent work by Renigunta et al. showing that expression of uromodulin significantly increases the activity of ROMK2 channel through direct interaction and positive regulation of its delivery to the plasma membrane. Lack of uromodulin in *Umod* knock-out mice leads to significant upregulation of ROMK2 that results from a reduction of the channel amount at the plasma membrane and its increase in the vesicular pool (26). However, the specificity of this effect requires further investigation as ion transporters of downstream segments (NCC, alpha-ENaC) were also found to be significantly upregulated in uromodulin deficient mice (27).

Studies in *Umod* knock-out mice showed that uromodulin plays a defensive role against urinary tract infection (UTI) (28, 29). This function is due to its ability to bind to pathogens of the urinary tract, e.g. type 1-fimbriated *E. coli*, competing with their binding to uroplakins on the urothelium (30) and is mediated by its high-mannose moiety. Indeed, one the seven sites of glycosylation (Asn 274) retains a high-mannose chain (Figure 1a), a feature that is conserved throughout evolution and that likely depends on the protein primary structure and folding (18). Uromodulin also plays a role in preventing the formation of kidney stones. Several *in vitro* studies (31) complemented by *in vivo* investigations on a rat nephrolithiasis model (32) showed that uromodulin reduces the aggregation of calcium crystals. Moreover, lack of uromodulin in knock-out mice leads to the formation of calcium crystals in the kidneys and progressive renal calcification (33, 34). Uromodulin exerts its protective function acting synergistically with osteopontin, as shown in double knock-out mice (35).

Although supported by *in vivo* evidence in *Umod* knock-out mice, the relevance of uromodulin as a protective molecule against UTI and nephrolithiasis is still unclear, as individuals with extremely reduced uromodulin urinary level, as patients carrying *UMOD* mutations (see below), do not show increased rates of urinary tract infections or renal stone formation.

Finally, uromodulin has also been suggested to play a role in innate immunity of the kidney. Several *in vitro* studies demonstrated that it can bind to immunity related molecules as IgG,

complement 1q and TNF-alpha (36-38). Uromodulin can also act as a chemoattractant (39) and as a pro-inflammatory molecule, able to interact with and activate components of the immune system, including monocytes, neutrophils (39) and myeloid dendritic cells via Toll-like receptor 4 (TLR4) (40). Administration of uromodulin induces tubulo-interstitial nephritis in rabbits, rats and mice (41, 42). In the mouse, this is accompanied by the production of anti-uromodulin antibodies that is dependent on TLR4 function (40). Taken together, these data suggest that uromodulin may act as a danger-signalling molecule, able to elicit an inflammatory response following conditions that damage the nephron integrity and lead to uromodulin release in the interstitial space. This hypothesis is supported by the evidence of interstitial uromodulin release associated with inflammatory cell infiltrate as well as of increased uromodulin-specific autoantibodies in several inflammatory disorders and infections of the urinary tract (43). However, the pro-inflammatory role of uromodulin remains controversial and has been recently challenged by El-Achkar et al. who showed that mice lacking uromodulin develop more functional and histological renal damage after ischemia-reperfusion injury compared with wild type animals, suggesting that uromodulin has a protective role against inflammation and necrosis induced by ischemic damage (44).

### Uromodulin-associated kidney diseases

Mutations in the *UMOD* gene cause medullary cystic kidney disease type 2 (MCKD2; MIM 603860) and familial juvenile hyperuricemic nephropathy (FJHN; MIM 162000) that are autosomal dominant tubulointerstitial kidney diseases. Being allelic disorders, MCKD2 and FJHN are collectively referred to as uromodulin-associated kidney disease (UAKD) (45). UAKD is a rare disorder. About fifty mutations have been reported so far (see below) and its prevalence is estimated to be 1/100,000 ([www.orphanet.org](http://www.orphanet.org)). The earliest symptom in UAKD patients is often hyperuricemia that results from reduced fractional excretion of uric acid, is present in about 80% of

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

patients and is frequently associated with gout (45, 46). Mild urine concentrating ability is an almost constant finding, sometimes resulting in polyuria and polydipsia (47). Chronic renal failure generally occurs between the second and fourth decade of life, although a significant intra- and inter-familial variability has been observed. At the histological analysis, UAKD is characterised by diffuse tubulointerstitial fibrosis with moderate inflammatory cell infiltrate and tubular atrophy (47, 48). Renal cysts (generally measuring 0,5 to 3 cm in diameter) are sometimes detected, mainly at the cortico-medullary junction (47, 49, 50). There is no specific therapy other than correction of water and electrolyte imbalance that may occur. Hyperuricaemia can be effectively treated with allopurinol (51) or uricosuric drugs as benzbromarone (52). Dialysis followed by renal transplantation is the preferred approach for renal failure. Few follow-up data are available on transplanted patients and suggest that renal transplant can effectively cure UAKD (53).

Mutations in the *UMOD* gene were also reported in two families affected by a variant of glomerulocystic kidney disease (GCKD) (MIM 609886), resembling UAKD phenotype (48, 54). Patients showed marked dilation of Bowman's space in most glomeruli that was associated with hyperuricemia, severe impairment of urine concentrating ability and no evidence of diabetes. Interestingly, homozygosity for a *UMOD* mutation has been reported in 3 affected individuals from a Spanish consanguineous family (55). Homozygous individuals display a more severe phenotype in comparison to heterozygous members of the same family in terms of earlier onset of hyperuricemia and faster progression to end-stage renal disease.

The analysis of uromodulin in renal biopsies and urine samples from patients with UAKD revealed some key findings. Immunohistochemistry and immunofluorescence analysis showed the presence of large uromodulin intracellular aggregates in the cells lining the TAL (47, 48, 56, 57). These inclusions co-localise with endoplasmic reticulum (ER) markers, suggesting that mutations affect protein delivery to the plasma membrane (57, 58). These findings are consistent with the presence of fibrillar or amorphous material within expanded stacks of the ER and of hyperplastic bundles of

the ER at the ultrastructural analysis (49, 57, 59). Defective transport of mutant protein was also demonstrated by a significant decrease of the urinary excretion of uromodulin UAKD patients (47, 48, 57, 60). The extent of the reduction was not entirely due to the progressive loss of renal function, suggesting a possible dominant negative effect on the trafficking of wild type protein.

Similar clinical findings, i.e. tubulo-interstitial nephritis and hyperuricaemia, can be found associated with mutations in the gene encoding the transcription factor hepatocyte nuclear factor (HNF)-1beta (*TCF2*) (MIM 137920) (61) and in the *REN* gene encoding renin (MIM 613093) (62). Moreover, two additional loci have been mapped on chromosome 1q21 (MCKD1) (63) and p22.1-p21 (FJHN3) (64).

#### *In vitro studies*

To date 51 *UMOD* mutations have been published (Figure 2). All but three (in-frame deletions) are missense changes that affect in about 50% (28/51) of the cases one of the conserved cysteine residues. *UMOD* mutations are clustered (94%) in exons 3 and 4 encoding for the N-terminal half of the protein; only three mutations have been reported so far in exons 5 (C347G) and 7 (A461E, G488R) and affect residues within the ZP domain (65-67).

The majority of reported *UMOD* mutations are predicted to cause protein misfolding, either by directly affecting the disulphide bond pattern or by destabilising the structure of EGF-like domains. Consistent with an effect of mutations on protein folding, we demonstrated in different cellular models that mutant uromodulin isoforms are defective in trafficking to the plasma membrane and are retained in the ER (48, 68). The results of our *in vitro* studies on *UMOD* mutations were confirmed by other reports on different mutation panels and different cell lines (12, 57, 66), clearly showing a common effect for all *UMOD* mutations. Overall, these data indicate ER retention of mutant uromodulin as a key step in the pathogenesis of UAKD and point at these diseases as an

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

additional member of the known ER storage diseases (59). Interestingly, *in vitro* studies suggest the presence of two classes of *UMOD* mutations, according to the extent of mutant protein retention in the ER. At the moment, no clear genotype-phenotype could be established due to the small number of affected families and/or incomplete or heterogeneous available clinical data.

Although the analyses of *in vitro* models identified a common effect of all *UMOD* mutations, they did not allow the identification of potential pathogenetic events downstream of mutant uromodulin ER retention. Mutant uromodulin expression in cellular models was reported to lead to cell apoptosis that could be rescued by treatment with colchicine and sodium 4-Phenylbutyrate (69). However, evidence for a proteotoxic effect of mutant uromodulin expression has not been observed when using different kidney cell lines under basal or stress conditions ((12), our unpublished results). As the cellular models so far reported were ineffective in reproducing key cellular hallmarks of the disease, i.e. mutant uromodulin aggregation, ER membrane expansion and dilation, and were developed in cell lines not expressing endogenous uromodulin, it cannot be excluded that a fully differentiated TAL cell is needed to properly assess *in vitro* the effect of mutant protein expression.

*In vivo models*

Two *Umod* knock-out mouse models have been reported and extensively characterised. As previously mentioned, these animals are more susceptible to urinary tract infections and more prone to renal stone formation and nephrocalcinosis. However, animals lacking uromodulin show few if any signs of UAKD, consistent with a gain-of-function effect of *UMOD* mutations. Indeed, no interstitial fibrosis, inflammatory infiltrate and renal cysts were observed up to three years of age (70). *Umod* knock-out mice show mild urinary concentrating defect after water deprivation test that could be due to a defect in the TAL reabsorptive capacity (27). Recent data showing reduced amount of ROMK2 channel at the plasma membrane in these animals supports this hypothesis (26).

Noteworthy, no renal phenotype was described in a transgenic mouse model expressing C148W human mutant uromodulin, likely because of low expression of the transgene (71). A mouse expressing uromodulin variant A227T was obtained by ENU mutagenesis. *Umod*<sup>A227T</sup> mice show some features of UAKD as the presence of uromodulin aggregates in TAL cells, urine concentration ability defect and reduced fractional excretion of uric acid but lack any of the histopathological signs of the disease and have additional metabolic alterations (72).

We recently generated and characterised an *in vivo* model of UAKD, i.e. a transgenic mouse expressing mutant uromodulin (Tg<sup>*Umod*C147W</sup>) (58). The mutation introduced in the murine protein (C147W) corresponds to the human mutation C148W that we previously identified in UAKD patients and extensively characterised *in vitro* (48). The phenotype in Tg<sup>*Umod*C147W</sup> mice was compared to expression-matched transgenic mice for wild type protein (Tg<sup>*Umod*wt</sup>). Tg<sup>*Umod*C147W</sup> mice specifically show progressive signs of renal damage, i.e. tubulo-interstitial fibrosis with inflammatory cell infiltration and tubule dilation. Interestingly, necrotic cells but no apoptosis were detected in distal tubules. Similarly to UAKD patients, Tg<sup>*Umod*C147W</sup> mice show urinary concentrating defect of renal origin that is present in young animals (12 weeks of age) and precedes renal failure. ER retention of mutant uromodulin precedes all other features starting at 1 week of age (our unpublished results) and progressing to the formation of massive intracellular aggregates and hyperplasia of ER membranes in 24 weeks-old kidneys. Tg<sup>*Umod*C147W</sup> mice hence recapitulate most of the disease features with the exception of hyperuricemia, likely because mice express urate oxidase, an enzyme that catalyses urate to allantoin conversion and that is absent in primates. We believe that the different phenotype in *Umod*<sup>A227T</sup> and Tg<sup>*Umod*C147W</sup> mouse models could be ascribed to the different genetic backgrounds in the two models (C3H *versus* FVB), to the different expression level of total uromodulin or to the fact that the A227T variant was not associated with UAKD in patients.



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

On the bases of current knowledge, we envisage a model of UAKD pathogenesis in which the key primary event is ER accumulation of mutant uromodulin in the TAL cells that could have both a gain-of-function and a loss-of-function effect (Figure 3a). On the one hand, it leads to TAL functional and structural injury, as suggested by distal tubule cell necrosis in Tg<sup>UmodC147W</sup> mice. On the other hand, it reduces the amount of uromodulin entering the secretory pathway and reaching the apical membrane, also affecting the trafficking of the wild-type protein. This could affect the efficient delivery of ion transporters in the TAL segment, as suggested by reduced amount of ROMK at the apical plasma membrane in the *Umod* knock-out mouse (26). Loss of functional TAL segment could be at the bases of the urinary concentrating defect in UAKD and lead to hyperuricaemia, likely as a secondary effect of increased compensatory sodium uptake in the proximal tubule (59), through a mechanism similar to the one leading to hyperuricaemia following chronic loop diuretic administration (73). The inflammatory process in the kidney of Tg<sup>UmodC147W</sup> mice could be triggered by ER stress pathways activated in TAL cells and/or by TAL cell necrosis eventually resulting in progressive interstitial fibrosis and tissue scarring, a final common pathway for many acute and chronic kidney injuries. Although we did not detect significant evidence of interstitial uromodulin we cannot exclude the possibility that inflammation could be enhanced by basolateral release of mutant uromodulin. An increase of uromodulin in the serum, possibly as a consequence of its interstitial release, was indeed reported in some UAKD patients (12) and could lead to the induction of pro-inflammatory cytokines (74). Renal cysts in UAKD could be a consequence of progressive TAL cellular damage and secondary proliferation. Recent findings showing the presence of uromodulin in primary cilia and a significant reduction of ciliary uromodulin in UAKD patients suggest the possibility that cysts could be related to cilia dysfunction (75). However, this seems unlikely taking into account the lack of renal cysts in *Umod* knock-out mice and recent evidence that strong *Umod* gene down-regulation is not sufficient to induce cystogenesis following HNF-1beta inactivation in the mouse (76).



Further studies will be needed in order to address this and other key open questions that would have relevance for potential therapeutic strategies, as the identification of the stress pathways that are activated by mutant uromodulin ER retention, the molecular basis of inflammation and fibrosis and the contribution of each factor in disease progression.

### **Uromodulin involvement in other pathological conditions of the kidney**

Several studies reported association of uromodulin abnormalities with pathologic conditions of the kidney. This includes accumulation of uromodulin in cast nephropathy and presence of protein deposits in the renal interstitium in reflux nephropathy, rejecting renal allografts and interstitial diseases (77-80). These deposits are sometimes associated with inflammatory infiltrate. Additionally, uromodulin urinary excretion is positively correlated with the estimated glomerular filtration rate (eGFR) and is reduced in several conditions affecting kidney function and/or integrity including glomerulonephritis, diabetes nephropathy, lupus nephritis, tubulointerstitial nephropathy and polycystic kidney disease (81). The use of urinary uromodulin as a diagnostic marker of renal disease has been recently questioned in an analysis of uromodulin urinary levels in 77 patients with chronic kidney disease (CKD) showing that 22% of the analysed patients have normal urinary levels of uromodulin (74). Qualitative changes in uromodulin processing have also been reported. The presence of shorter abnormally processed urinary uromodulin fragments was observed in Fabry disease patients (82). Interestingly, shorter uromodulin fragments were significantly decreased following enzyme replacement therapy.

### **Uromodulin in genome-wide association studies**

Genome-wide association studies (GWAS) have successfully identified genomic loci containing

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

susceptibility variants associated with the risk of complex traits and markers of renal function ((83), for review). In particular, common variants in the *UMOD* gene have been associated with the risk of CKD, eGFR and other complex traits such as kidney stones and hypertension (Table 1, Figure 2).

The first GWAS on CKD, conducted in ~20000 participants of European ancestry from unselected, population-based cohorts from the CHARGE consortium, identified a top SNP (rs12917707) located 3.4 kb upstream of *UMOD* associated with the risk of CKD. The minor T allele of rs12917707 was associated with a 20% reduction in the risk of CKD, and the association was independent of major kidney disease risk factors including older age, male gender, and presence of hypertension or diabetes. Furthermore, prospective information from the ARIC study (n=952 cases) demonstrated that the T allele of rs12917707 was associated with a lower relative hazard of incident CKD over ~15 years of follow-up (HR 0.81, 95% CI= 0.72-0.92) (84). The strong association of rs12917707 with CKD was supported by a subsequent analysis performed in the larger cohort of the CKDGen consortium (85). It is important to note that the rs12917707 variant of *UMOD* was also associated with two indices of renal function, eGFR<sub>crea</sub> and eGFR<sub>cys</sub>, based on serum creatinine and serum cystatin C respectively (84, 85). The rs12917707 variant of *UMOD* was associated with both higher eGFR<sub>cys</sub> and eGFR<sub>creat</sub> and lower risk of developing CKD, consistent with a protective effect (84, 85). As expected, the variants described above explain a small percentage (typically, less than 1%) of the variance in eGFR<sub>crea</sub>. Of note, there was no significant association of rs12917707 with hyperuricemia and gout (84).

An independent replication of the findings of Köttgen et al. was provided by Gudbjartsson et al. (86) who found that the SNP rs4293393, located 300 bp upstream of *UMOD*, is associated with increased risk of CKD and elevated serum creatinine (sCreat) in a large Icelandic population. The rs4293393 variant was also associated with increased serum levels of uric acid and increased risk of gout, contrasting with a *lower* risk of formation of calcium-containing kidney stones in the Icelandic population. The association of the rs4293393 and rs12917707 variants with serum

creatinine levels was confirmed in a meta-analysis of five European isolates (EUROSPAN) (87) and in the large European cohort reported by Chambers et al. (88). Of note, the rs4293393 variant is in perfect linkage disequilibrium (LD) in the HapMap CEU (89) with the rs12917707 variant ( $D' = 1$ ;  $r^2 = 1$ ) (Figure 2).

Recently, the Global BPGen consortium used an extreme case-control design to identify a locus in the 5' region of *UMOD* (rs13333226) associated with hypertension (90). The minor G allele of rs13333226 was associated with a lower risk of hypertension (OR: 0.6; 95% CI= 0.5-0.73), with each copy of the G allele being associated with 0.49 mmHg lower SBP and 0.30 mmHg DBP. The minor allele of rs13333226 was also associated with eGFR, but adjustment for this variable in a subset of 13466 individuals confirmed the association of rs13333226 with lower risk for hypertension. The rs13333226 variant of *UMOD* was also associated with long-term cardiovascular outcomes among the 26654 subjects from the Swedish population-based MDC study: each copy of the G allele associated with a 7.7% reduction in risk of cardiovascular disease after adjusting for age, sex, BMI and smoking status followed-up for 12 years. The rs13333226 variant is in complete LD with rs4293393 and rs12917707 variants ( $D' = 1$ ,  $r^2 = 1$ , HapMap CEU) (Figure 2).

Typically, GWAS yield loci that are statistically associated with a quantitative trait or a disease state. In an effort to evaluate the functional link between variants in *UMOD*, the level of uromodulin in the urine and the risk of developing CKD, Köttgen et al. performed a nested case-control study (n=200) of incident CKD (followed-up for 9.9 years) within the Framingham Heart Study (91). They showed that baseline urinary uromodulin levels were 51% higher in CKD than controls and that the protective C allele of rs4293393 was associated with *lower* urinary uromodulin levels and higher eGFR, in a dose-dependent manner (91). **The perfectly correlated** minor G allele of rs13333226 (which is protective against hypertension) was associated with lower urinary excretion of uromodulin in a subset of participants of the population-based HERCULES study (n=110) and hypertensive individuals from the BRIGHT study (n=256), with a potential relation

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

with lower fractional excretion of sodium and lower endogenous lithium clearance (90). The minor T allele of rs12917707 that is protective for CKD, was also shown to be associated with lower urinary uromodulin among coronary artery disease (CAD) patients (n=120) from the Heart and Soul Study (92). However, in these patients uromodulin urinary levels were not associated with CKD, suggesting that different mechanisms could be responsible for kidney function decline in patients with and without CAD. With the limitation of small sample size and complexity of the factors (age, renal function, diet, drugs, etc.) influencing the excretion of uromodulin, these studies (i) point to the potential of uromodulin as a biomarker for CKD; and (ii) suggest that higher urinary excretion of uromodulin may be deleterious and precede the development of CKD and/or hypertension (Figure 3b).

Future studies should confirm the potential association between *UMOD* variants and blood pressure (as a continuous trait) and investigate the causality of the variants described above, which are all in strong LD in the 5' region of *UMOD*, or determine causal variants by resequencing. They should also characterise the factors influencing the urinary excretion of uromodulin in large cohorts, as well as the function, regulation and functional interactions of uromodulin in the epithelial cells lining the TAL in the human kidney (93). The transgenic and knock-out mouse models described above will undoubtedly be useful to analyse the role of uromodulin and distinct between primary effects or functional adaptations caused by its deletion or overexpression in the kidney.

**Conclusions and perspectives**

In the past years uromodulin has been rediscovered thanks to key findings from multidisciplinary studies that ranged from *in vitro* studies, genetic analysis in Mendelian disorders, characterisation of different mouse models and genome-wide association studies. This has led to a scientific Renaissance of the field that will boost future investigations aimed at understanding the precise role

of uromodulin in the TAL and its potential link with ion transport and innate immunity of the kidney, the molecular mechanisms that regulate its expression and secretion, the significance of its basolateral and urinary release and its use as a biomarker. Moreover, studies on uromodulin-associated kidney diseases will help understanding the pathophysiology of the TAL segment and will take advantage of pre-clinical models to identify potential therapeutic strategies. Finally, the association of uromodulin with CKD and hypertension will need further investigation to clarify the biological effect of the identified risk variants and to assess for the presence of additional linked variants that may have a causal role.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**DISCLOSURE**

None to declare.

For Peer Review Only

## REFERENCES

1. Tamm I, Horsfall FL. Characterisation and separation of an inhibitor of viral hemagglutination present in urine. *Proc Soc Exp Biol Med* 1950; 108-114.
2. Kumar S, Muchmore A. Tamm-Horsfall protein--uromodulin (1950-1990). *Kidney Int* 1990; **37**: 1395-1401.
3. Muchmore AV, Decker JM. Uromodulin: a unique 85-kilodalton immunosuppressive glycoprotein isolated from urine of pregnant women. *Science* 1985; **229**: 479-481.
4. Pennica D, Kohr WJ, Kuang WJ, *et al.* Identification of human uromodulin as the Tamm-Horsfall urinary glycoprotein. *Science* 1987; **236**: 83-88.
5. Serafini-Cessi F, Malagolini N, Hoops TC, *et al.* Biosynthesis and oligosaccharide processing of human Tamm-Horsfall glycoprotein permanently expressed in HeLa cells. *Biochem Biophys Res Commun* 1993; **194**: 784-790.
6. Easton RL, Patankar MS, Clark GF, *et al.* Pregnancy-associated changes in the glycosylation of tamm-horsfall glycoprotein. Expression of sialyl Lewis(x) sequences on core 2 type O-glycans derived from uromodulin. *J Biol Chem* 2000; **275**: 21928-21938.
7. Jovine L, Qi H, Williams Z, *et al.* The ZP domain is a conserved module for polymerization of extracellular proteins. *Nat Cell Biol* 2002; **4**: 457-461.
8. Wiggins RC. Uromucoid (Tamm-Horsfall glycoprotein) forms different polymeric arrangements on a filter surface under different physicochemical conditions. *Clin Chim Acta* 1987; **162**: 329-340.
9. Bachmann S, Metzger R, Bunnemann B. Tamm-Horsfall protein-mRNA synthesis is localized to the thick ascending limb of Henle's loop in rat kidney. *Histochemistry* 1990; **94**: 517-523.
10. Bachmann S, Koeppen-Hagemann I, Kriz W. Ultrastructural localization of Tamm-Horsfall glycoprotein (THP) in rat kidney as revealed by protein A-gold immunocytochemistry. *Histochemistry* 1985; **83**: 531-538.
11. Peach RJ, Day WA, Ellingsen PJ, *et al.* Ultrastructural localization of Tamm-Horsfall protein in human kidney using immunogold electron microscopy. *Histochem J* 1988; **20**: 156-164.
12. Jennings P, Aydin S, Kotanko P, *et al.* Membrane targeting and secretion of mutant uromodulin in familial juvenile hyperuricemic nephropathy. *J Am Soc Nephrol* 2007; **18**: 264-273.
13. Chen YT, Kobayashi A, Kwan KM, *et al.* Gene expression profiles in developing nephrons using Lim1 metanephric mesenchyme-specific conditional mutant mice. *BMC Nephrol* 2006; **7**: 1.
14. Jouret F, Debaix H, Devuyst O. A catalog of gene expression in the developing kidney. *Kidney Int* 2004; **66**: 867-868.
15. Zimmerhackl LB, Rostasy K, Wiegele G, *et al.* Tamm-Horsfall protein as a marker of tubular maturation. *Pediatr Nephrol* 1996; **10**: 448-452.
16. Chabardes-Garonne D, Mejean A, Aude JC, *et al.* A panoramic view of gene expression in the human kidney. *Proc Natl Acad Sci U S A* 2003; **100**: 13710-13715.
17. Grant AM, Neuberger A. The turnover rate of rabbit urinary Tamm-Horsfall glycoprotein. *Biochem J* 1973; **136**: 659-668.
18. Serafini-Cessi F, Malagolini N, Cavallone D. Tamm-Horsfall glycoprotein: biology and clinical relevance. *Am J Kidney Dis* 2003; **42**: 658-676.
19. Santambrogio S, Cattaneo A, Bernascone I, *et al.* Urinary uromodulin carries an intact ZP domain generated by a conserved C-terminal proteolytic cleavage. *Biochem Biophys Res Commun* 2008; **370**: 410-413.
20. Schaeffer C, Santambrogio S, Perucca S, *et al.* Analysis of uromodulin polymerization provides new insights into the mechanisms regulating ZP domain-mediated protein assembly. *Mol Biol Cell* 2009; **20**: 589-599.
21. Jovine L, Qi H, Williams Z, *et al.* A duplicated motif controls assembly of zona pellucida



domain proteins. *Proc Natl Acad Sci U S A* 2004; **101**: 5922-5927.

22. Kistler AD, Mischak H, Poster D, *et al.* Identification of a unique urinary biomarker profile in patients with autosomal dominant polycystic kidney disease. *Kidney Int* 2009; **76**: 89-96.

23. Yu S, Lowe AW. The pancreatic zymogen granule membrane protein, GP2, binds *Escherichia coli* Type 1 fimbriae. *BMC Gastroenterol* 2009; **9**: 58.

24. Howie AJ, Lote CJ, Cunningham AA, *et al.* Distribution of immunoreactive Tamm-Horsfall protein in various species in the vertebrate classes. *Cell Tissue Res* 1993; **274**: 15-19.

25. Ying WZ, Sanders PW. Dietary salt regulates expression of Tamm-Horsfall glycoprotein in rats. *Kidney Int* 1998; **54**: 1150-1156.

26. Renigunta A, Renigunta V, Saritas T, *et al.* Tamm-Horsfall glycoprotein interacts with renal outer medullary potassium channel ROMK2 and regulates its function. *J Biol Chem* 2010.

27. Bachmann S, Mutig K, Bates J, *et al.* Renal effects of Tamm-Horsfall protein (uromodulin) deficiency in mice. *Am J Physiol Renal Physiol* 2005; **288**: F559-567.

28. Bates JM, Raffi HM, Prasad K, *et al.* Tamm-Horsfall protein knockout mice are more prone to urinary tract infection: rapid communication. *Kidney Int* 2004; **65**: 791-797.

29. Mo L, Zhu XH, Huang HY, *et al.* Ablation of the Tamm-Horsfall protein gene increases susceptibility of mice to bladder colonization by type 1-fimbriated *Escherichia coli*. *Am J Physiol Renal Physiol* 2004; **286**: F795-802.

30. Pak J, Pu Y, Zhang ZT, *et al.* Tamm-Horsfall protein binds to type 1 fimbriated *Escherichia coli* and prevents *E. coli* from binding to uroplakin Ia and Ib receptors. *J Biol Chem* 2001; **276**: 9924-9930.

31. Chen WC, Lin HS, Tsai FJ, *et al.* Effects of Tamm-Horsfall protein and albumin on the inhibition of free radicals. *Urol Int* 2001; **67**: 305-309.

32. Miyake O, Yoshioka T, Yoshimura K, *et al.* Expression of Tamm-Horsfall protein in stone-forming rat models. *Br J Urol* 1998; **81**: 14-19.

33. Mo L, Huang HY, Zhu XH, *et al.* Tamm-Horsfall protein is a critical renal defense factor protecting against calcium oxalate crystal formation. *Kidney Int* 2004; **66**: 1159-1166.

34. Liu Y, Mo L, Goldfarb DS, *et al.* Progressive renal papillary calcification and ureteral stone formation in mice deficient for Tamm-Horsfall protein. *Am J Physiol Renal Physiol* 2010; **299**: F469-478.

35. Mo L, Liaw L, Evan AP, *et al.* Renal calcinosis and stone formation in mice lacking osteopontin, Tamm-Horsfall protein, or both. *Am J Physiol Renal Physiol* 2007; **293**: F1935-1943.

36. Rhodes DC, Hinsman EJ, Rhodes JA. Tamm-Horsfall glycoprotein binds IgG with high affinity. *Kidney Int* 1993; **44**: 1014-1021.

37. Rhodes DC. Binding of Tamm-Horsfall protein to complement 1q measured by ELISA and resonant mirror biosensor techniques under various ionic-strength conditions. *Immunol Cell Biol* 2000; **78**: 474-482.

38. Hession C, Decker JM, Sherblom AP, *et al.* Uromodulin (Tamm-Horsfall glycoprotein): a renal ligand for lymphokines. *Science* 1987; **237**: 1479-1484.

39. Schmid M, Prajczek S, Gruber LN, *et al.* Uromodulin facilitates neutrophil migration across renal epithelial monolayers. *Cell Physiol Biochem* 2010; **26**: 311-318.

40. Saemann MD, Weichhart T, Zeyda M, *et al.* Tamm-Horsfall glycoprotein links innate immune cell activation with adaptive immunity via a Toll-like receptor-4-dependent mechanism. *J Clin Invest* 2005; **115**: 468-475.

41. Mayrer AR, Kashgarian M, Ruddle NH, *et al.* Tubulointerstitial nephritis and immunologic responses to Tamm-Horsfall protein in rabbits challenged with homologous urine or Tamm-Horsfall protein. *J Immunol* 1982; **128**: 2634-2642.

42. Hoyer JR. Tubulointerstitial immune complex nephritis in rats immunized with Tamm-Horsfall protein. *Kidney Int* 1980; **17**: 284-292.

43. Saemann MD, Weichhart T, Horl WH, *et al.* Tamm-Horsfall protein: a multilayered defence



molecule against urinary tract infection. *Eur J Clin Invest* 2005; **35**: 227-235.

44. El-Achkar TM, Wu XR, Rauchman M, *et al.* Tamm-Horsfall protein protects the kidney from ischemic injury by decreasing inflammation and altering TLR4 expression. *Am J Physiol Renal Physiol* 2008; **295**: F534-544.

45. Hart TC, Gorry MC, Hart PS, *et al.* Mutations of the UMOD gene are responsible for medullary cystic kidney disease 2 and familial juvenile hyperuricaemic nephropathy. *J Med Genet* 2002; **39**: 882-892.

46. Bleyer AJ, Hart TC. Familial juvenile hyperuricaemic nephropathy. *Qjm* 2003; **96**: 867-868.

47. Dahan K, Devuyst O, Smaers M, *et al.* A cluster of mutations in the UMOD gene causes familial juvenile hyperuricemic nephropathy with abnormal expression of uromodulin. *J Am Soc Nephrol* 2003; **14**: 2883-2893.

48. Rampoldi L, Caridi G, Santon D, *et al.* Allelism of MCKD, FJHN and GCKD caused by impairment of uromodulin export dynamics. *Hum Mol Genet* 2003; **12**: 3369-3384.

49. Nasr SH, Lucia JP, Galgano SJ, *et al.* Uromodulin storage disease. *Kidney Int* 2008; **73**: 971-976.

50. Scolari F, Ghiggeri GM, Casari G, *et al.* Autosomal dominant medullary cystic disease: a disorder with variable clinical pictures and exclusion of linkage with the NPH1 locus. *Nephrol Dial Transplant* 1998; **13**: 2536-2546.

51. Bleyer AJ, Zivna M, Kmoch S. Uromodulin-associated kidney disease. *Nephron Clin Pract* 2011; **118**: c31-36.

52. Lhotta K, Gruber J, Sgonc R, *et al.* Apoptosis of tubular epithelial cells in familial juvenile gouty nephropathy. *Nephron* 1998; **79**: 340-344.

53. Labriola L, in Dahan K, Pirson Y. Outcome of kidney transplantation in familial juvenile hyperuricaemic nephropathy. *Nephrol Dial Transplant* 2007; **22**: 3070-3073.

54. Lens XM, Banet JF, Outeda P, *et al.* A novel pattern of mutation in uromodulin disorders: autosomal dominant medullary cystic kidney disease type 2, familial juvenile hyperuricemic nephropathy, and autosomal dominant glomerulocystic kidney disease. *Am J Kidney Dis* 2005; **46**: 52-57.

55. Rezende-Lima W, Parreira KS, Garcia-Gonzalez M, *et al.* Homozygosity for uromodulin disorders: FJHN and MCKD-type 2. *Kidney Int* 2004; **66**: 558-563.

56. Bleyer AJ, Hart TC, Willingham MC, *et al.* Clinico-pathologic findings in medullary cystic kidney disease type 2. *Pediatr Nephrol* 2005; **20**: 824-827.

57. Vylet'al P, Kublova M, Kalbacova M, *et al.* Alterations of uromodulin biology: a common denominator of the genetically heterogeneous FJHN/MCKD syndrome. *Kidney Int* 2006; **70**: 1155-1169.

58. Bernascone I, Janas S, Ikehata M, *et al.* A transgenic mouse model for uromodulin-associated kidney diseases shows specific tubulo-interstitial damage, urinary concentrating defect and renal failure. *Hum Mol Genet* 2010; **19**: 2998-3010.

59. Scolari F, Caridi G, Rampoldi L, *et al.* Uromodulin storage diseases: clinical aspects and mechanisms. *Am J Kidney Dis* 2004; **44**: 987-999.

60. Bleyer AJ, Hart TC, Shihabi Z, *et al.* Mutations in the uromodulin gene decrease urinary excretion of Tamm-Horsfall protein. *Kidney Int* 2004; **66**: 974-977.

61. Bingham C, Ellard S, van't Hoff WG, *et al.* Atypical familial juvenile hyperuricemic nephropathy associated with a hepatocyte nuclear factor-1beta gene mutation. *Kidney Int* 2003; **63**: 1645-1651.

62. Zivna M, Hulkova H, Matignon M, *et al.* Dominant renin gene mutations associated with early-onset hyperuricemia, anemia, and chronic kidney failure. *Am J Hum Genet* 2009; **85**: 204-213.

63. Christodoulou K, Tsingis M, Stavrou C, *et al.* Chromosome 1 localization of a gene for autosomal dominant medullary cystic kidney disease. *Hum Mol Genet* 1998; **7**: 905-911.

64. Piret SE, Danoy P, Dahan K, *et al.* Genome-wide study of familial juvenile hyperuricaemic

- (gouty) nephropathy (FJHN) indicates a new locus, FJHN3, linked to chromosome 2p22.1-p21. *Hum Genet* 2011; **129**: 51-58.
65. Tinschert S, Ruf N, Bernascone I, *et al.* Functional consequences of a novel uromodulin mutation in a family with familial juvenile hyperuricaemic nephropathy. *Nephrol Dial Transplant* 2004; **19**: 3150-3154.
66. Williams SE, Reed AA, Galvanovskis J, *et al.* Uromodulin mutations causing familial juvenile hyperuricaemic nephropathy lead to protein maturation defects and retention in the endoplasmic reticulum. *Hum Mol Genet* 2009; **18**: 2963-2974.
67. Lee DH, Kim JK, Oh SE, *et al.* A case of familial juvenile hyperuricemic nephropathy with novel uromodulin gene mutation, a novel heterozygous missense mutation in Korea. *J Korean Med Sci* 2010; **25**: 1680-1682.
68. Bernascone I, Vavassori S, Di Pentima A, *et al.* Defective intracellular trafficking of uromodulin mutant isoforms. *Traffic* 2006; **7**: 1567-1579.
69. Choi SW, Ryu OH, Choi SJ, *et al.* Mutant tamm-horsfall glycoprotein accumulation in endoplasmic reticulum induces apoptosis reversed by colchicine and sodium 4-phenylbutyrate. *J Am Soc Nephrol* 2005; **16**: 3006-3014.
70. Raffi HS, Bates Jr JM, Laszik Z, *et al.* Tamm-Horsfall Protein Acts as a General Host-Defense Factor against Bacterial Cystitis. *Am J Nephrol* 2005; **25**: 570-578.
71. Takiue Y, Hosoyamada M, Yokoo T, *et al.* Production and characterization of transgenic mice harboring mutant human UMOD gene. *Nucleosides Nucleotides Nucleic Acids* 2008; **27**: 596-600.
72. Kemter E, Rathkolb B, Rozman J, *et al.* Novel missense mutation of uromodulin in mice causes renal dysfunction with alterations in urea handling, energy, and bone metabolism. *Am J Physiol Renal Physiol* 2009; **297**: F1391-1398.
73. Jackson EK. Diuretics, in: *Goodman & Gilman's the Pharmacological Basis of Therapeutics*, eds. Hardman J. G. LLE, Gilman A. G. McGraw-Hill: New York, 2001, pp 757-787.
74. Prajczek S, Heidenreich U, Pfaller W, *et al.* Evidence for a role of uromodulin in chronic kidney disease progression. *Nephrol Dial Transplant* 2010; **In Press**.
75. Zaucke F, Boehnlein JM, Steffens S, *et al.* Uromodulin is expressed in renal primary cilia and UMOD mutations result in decreased ciliary uromodulin expression. *Hum Mol Genet* 2010.
76. Verdeguer F, Le Corre S, Fischer E, *et al.* A mitotic transcriptional switch in polycystic kidney disease. *Nat Med* 2010; **16**: 106-110.
77. Resnick JS, Sisson S, Vernier RL. Tamm-Horsfall protein. Abnormal localization in renal disease. *Lab Invest* 1978; **38**: 550-555.
78. Zager RA, Cotran RS, Hoyer JR. Pathologic localization of Tamm-Horsfall protein in interstitial deposits in renal disease. *Lab Invest* 1978; **38**: 52-57.
79. Cotran RS, Galvanek E. Immunopathology of human tubulo-interstitial diseases: localization of immunoglobulins complement and Tamm-Horsfall protein. *Contrib Nephrol* 1979; **16**: 126-131.
80. Cohen AH, Border WA, Rajfer J, *et al.* Interstitial Tamm-Horsfall protein in rejecting renal allografts. Identification and morphologic pattern of injury. *Lab Invest* 1984; **50**: 519-525.
81. Vyletal P, Bleyer AJ, Kmoch S. Uromodulin Biology and Pathophysiology - An Update. *Kidney Blood Press Res* 2010; **33**: 456-475.
82. Vyletal P, Hulkova H, Zivna M, *et al.* Abnormal expression and processing of uromodulin in Fabry disease reflects tubular cell storage alteration and is reversible by enzyme replacement therapy. *J Inherit Metab Dis* 2008; **31**: 508-517.
83. Kottgen A. Genome-wide association studies in nephrology research. *Am J Kidney Dis* 2010; **56**: 743-758.
84. Kottgen A, Glazer NL, Dehghan A, *et al.* Multiple loci associated with indices of renal function and chronic kidney disease. *Nat Genet* 2009.
85. Kottgen A, Pattaro C, Boger CA, *et al.* New loci associated with kidney function and chronic kidney disease. *Nat Genet* 2010; **42**: 376-384.

86. Gudbjartsson DF, Holm H, Indridason OS, *et al.* Association of variants at UMOD with chronic kidney disease and kidney stones-role of age and comorbid diseases. *PLoS Genet* 2010; **6**: e1001039.
87. Pattaro C, De Grandi A, Vitart V, *et al.* A meta-analysis of genome-wide data from five European isolates reveals an association of COL22A1, SYT1, and GABRR2 with serum creatinine level. *BMC Med Genet* 2010; **11**: 41.
88. Chambers JC, Zhang W, Lord GM, *et al.* Genetic loci influencing kidney function and chronic kidney disease. *Nat Genet* 2010; **42**: 373-375.
89. Consortium TIH. A haplotype map of the human genome. *Nature* 2005; **437**: 1299-1320.
90. Padmanabhan S, Melander O, Johnson T, *et al.* Genome-wide association study of blood pressure extremes identifies variant near UMOD associated with hypertension. *PLoS Genet* 2010; **6**: e1001177.
91. Kottgen A, Hwang SJ, Larson MG, *et al.* Uromodulin levels associate with a common UMOD variant and risk for incident CKD. *J Am Soc Nephrol* 2010; **21**: 337-344.
92. Shlipak MG, Li Y, Fox C, *et al.* Uromodulin concentrations are not associated with incident CKD among persons with coronary artery disease. *BMC Nephrol* 2011; **12**: 2.
93. Belge H, Gailly P, Schwaller B, *et al.* Renal expression of parvalbumin is critical for NaCl handling and response to diuretics. *Proc Natl Acad Sci U S A* 2007; **104**: 14849-14854.
94. Han L, Monne M, Okumura H, *et al.* Insights into egg coat assembly and egg-sperm interaction from the X-ray structure of full-length ZP3. *Cell* 2010; **143**: 404-415.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**TITLES AND LEGENDS**

**Figure 1.** (a) The predicted structure of uromodulin contains a leader peptide (predicted to be cleaved at residue 23), three EGF-like domains (EGF-II and EGF-III are calcium-binding), a central domain of unknown function (named D8C as it contains 8 conserved cysteines), a zona pellucida (ZP) domain and a glycosylphosphatidylinositol (GPI)-anchoring site (predicted at position 614). The seven N-glycosylation sites are indicated. The high-mannose chain on residue Asn 274 is shown in red. (b) Model of uromodulin maturation, excretion and polymerisation. Uromodulin is synthesised in TAL tubular epithelial cells. It is co-translationally inserted in the ER where GPI-anchoring, formation of intramolecular disulphide bonds and N-glycosylation take place. In the Golgi, all glycan chains are modified with the exception of the one on Asn 274 that retains a high-mannose moiety. Uromodulin reaches the plasma membrane in a polymerisation-incompetent conformation kept by the interaction of two hydrophobic motifs (red), one within the ZP domain (Internal Hydrophobic Patch) and one localised between the ZP domain and the GPI-anchoring site (External Hydrophobic Patch). Proteolytic cleavage by a yet to be identified protease (scissors) releases the hydrophobic interaction generating a polymerisation-competent monomer that is assembled into polymeric filaments. The orientation of uromodulin monomers within a filament is hypothetical and deduced from structural data on ZP3 protein (94).

**Figure 2.** Summary of uromodulin genetic variants associated with chronic diseases of the kidney. Upper panel: all published uromodulin mutations in UAKD patients are shown relative to their localisation and effect in the protein sequence. Fifty-one mutations have been reported to date: 29 (57%) affect or introduce cysteine residues directly altering the disulphide bonds pattern; 19 (37%) are missense changes affecting residues other than cysteine; 3 (6%) are in-frame deletions. Middle panel: exon/intron structure of the human *UMOD* gene. Coding parts are shown in blue. Most of *UMOD* mutations are clustered in exons 3 and 4. Lower panel: the position of top SNPs that were

identified in different GWAS is shown (red). These variants are within the same linkage disequilibrium block spanning *UMOD* promoter to exon 7, as shown by the linkage disequilibrium plot ( $r^2$  values, adapted from Haploview 4.2 output, data from HapMap CEU, release #28).

**Figure 3.** Uromodulin and chronic diseases of the kidney. (a) In UAKD, mutations in *UMOD* cause ER retention and aggregation of mutant protein. This leads to TAL dysfunction and urinary concentrating defect and to a dramatic decrease of uromodulin urinary levels, suggesting a dominant negative effect on the transport of wild type uromodulin to the plasma membrane. This effect could also affect the delivery of other proteins, e.g. ion transporters, on the plasma membrane contributing to TAL dysfunction. Through a still unknown mechanism, intracellular retention eventually results in TAL cell damage and necrosis leading to fibrosis and inflammatory cell infiltrate. Inflammation could be enhanced by basolateral release of uromodulin. (b) GWAS studies identified *UMOD* variants that are associated with eGFR and increased risk of CKD, hypertension and cardiovascular disease. These variants are associated with increased urinary uromodulin levels that could be due to increased uromodulin expression, decreased membrane-anchoring efficiency, faster protein sorting to the apical membrane or increased proteolytic release. The causal relationship between *UMOD* variants, increased urinary uromodulin and development of CKD, hypertension or cardiovascular disease is presently unknown.

Table 1. *UMOD* variants and traits identified by GWAS

Top SNP	Position <sup>a</sup>	Trait	Consortium <sup>d</sup>	Discovery cohort (n)	Replication (n)	Reference
rs12917707	-3403 bp	CKD <sup>b</sup>	CHARGE	19877	21466	(84)
rs12917707	-3403 bp	CKD <sup>b</sup>	CKDGen	62237	NA	(85)
rs12917707	-3403 bp	eGFR <sub>crea</sub>	CHARGE	18127	21466	(84)
rs12917707	-3403 bp	eGFR <sub>cys</sub>	CHARGE	12266	NA	(84)
rs12917707	-3403 bp	eGFR <sub>crea</sub>	CKDGen	67093	NA	(85)
rs12917707	-3403 bp	eGFR <sub>cys</sub>	CKDGen	20957	NA	(85)
rs4293393	-300 bp	sCreat	EUROSPAN <sup>e</sup>	4006	NA	(87)
rs12917707	-3403 bp	sCreat	Nine studies <sup>f</sup>	23090	16626	(88)
rs4293393	-300 bp	CKD	Iceland	2903 vs. 35818 <sup>g</sup>	300 vs. 2964 <sup>g</sup>	(86)
rs4293393	-300 bp	sCreat	Iceland	22256	2,379	(86)
rs4293393	-300 bp	sUrate	Iceland	6583	NA	(86)
rs4293393	-300 bp	sUrea	Iceland	4084	NA	(86)
rs4293393	-300 bp	Kidney stones	Iceland	1689 vs. 37076 <sup>g</sup>	1972 vs. 6125 <sup>g,h</sup>	(86)
rs13333226	-1867 bp	Hypertension <sup>c</sup>	Global BPgen	1621 vs. 1699 <sup>g</sup>	19845 vs. 1654 <sup>g</sup>	(90)

Footnote: The frequency of the minor allele for rs12917707, rs13333226 and rs4293393 is ~ 0.18. All the indicated SNPs are in complete linkage disequilibrium ( $D'=1$ ,  $r^2=1$ ; HapMap CEU, release #28).

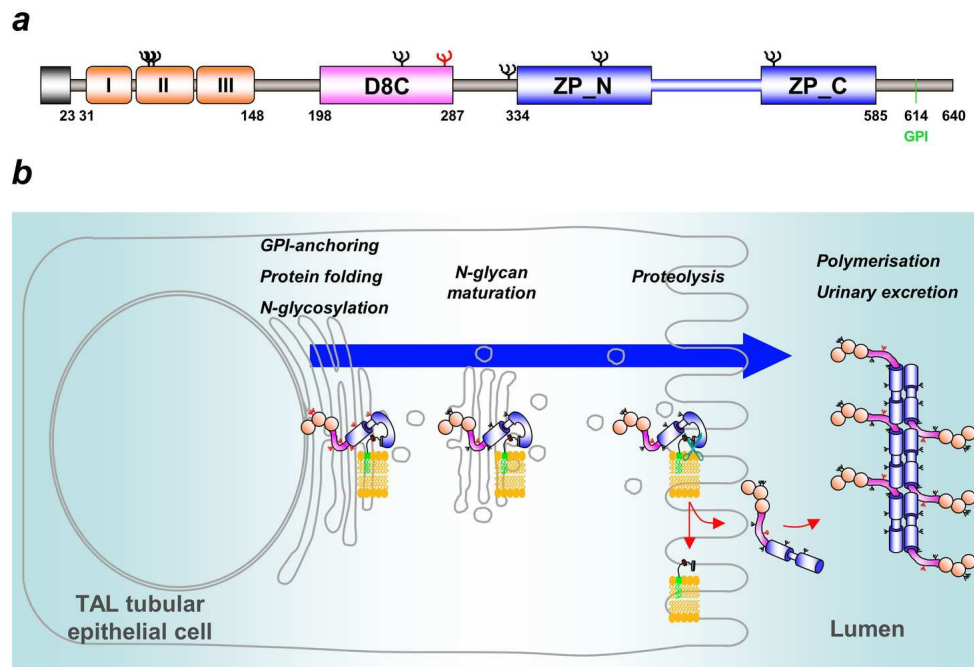
<sup>a</sup>Position relative to *UMOD* transcription start site (UCSC Genome Browser, GRCh37); <sup>b</sup>CKD definition: eGFR< 60mL/min/1.73m<sup>2</sup> using the MDRD equation;

<sup>c</sup>Hypertension defined as at least two consecutive blood pressure measurements of  $\geq 160$  mmHg systolic and  $\geq 100$  mmHg diastolic , with diagnosis made before age 63 years;

<sup>d</sup>All cohorts are from European descent; <sup>e</sup>EUROSPAN is a combination of five European genetic isolates; <sup>f</sup>European participants from the following studies: LOLIPOP, CoLaus, SardiNIA, TwinsUK, BRIGHT, Fenland, NFBC1966, NESDA, InChianti; <sup>g</sup>cases vs. controls; <sup>h</sup>combined replication on Icelandic and Dutch subjects.

Abbreviations: eGFR<sub>crea</sub>: estimated GFR based on serum creatinine; eGFR<sub>cys</sub>, estimated GFR based on serum cystatin C; sCreat, serum creatinine; CKD, chronic kidney disease; NA, not available.





63x46mm (600 x 600 DPI)

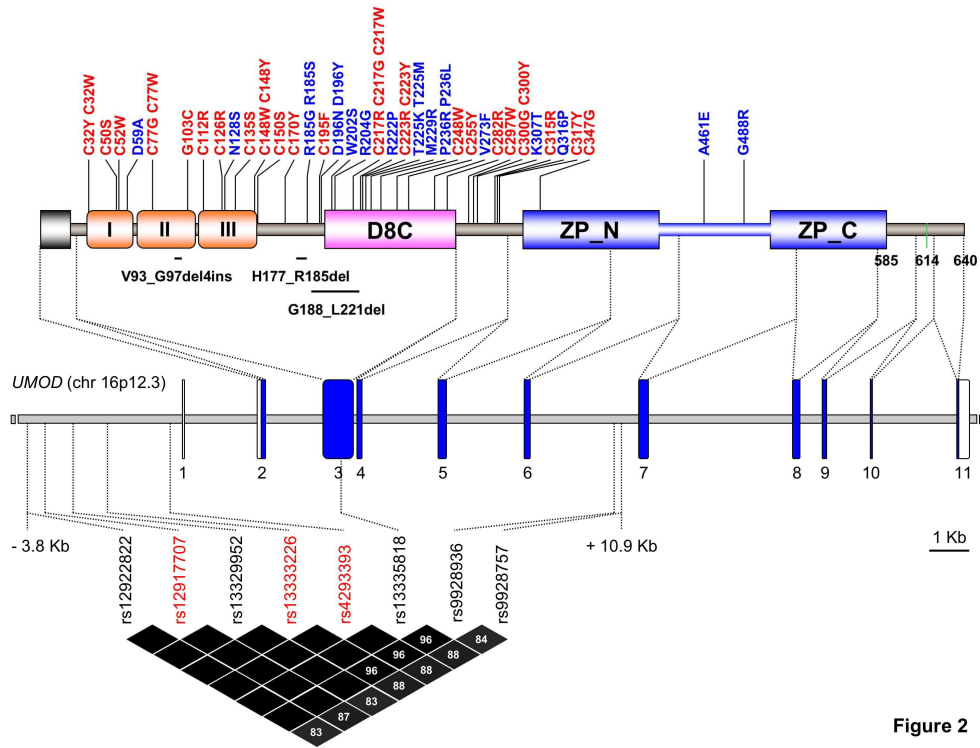


Figure 2

177x133mm (428 x 428 DPI)



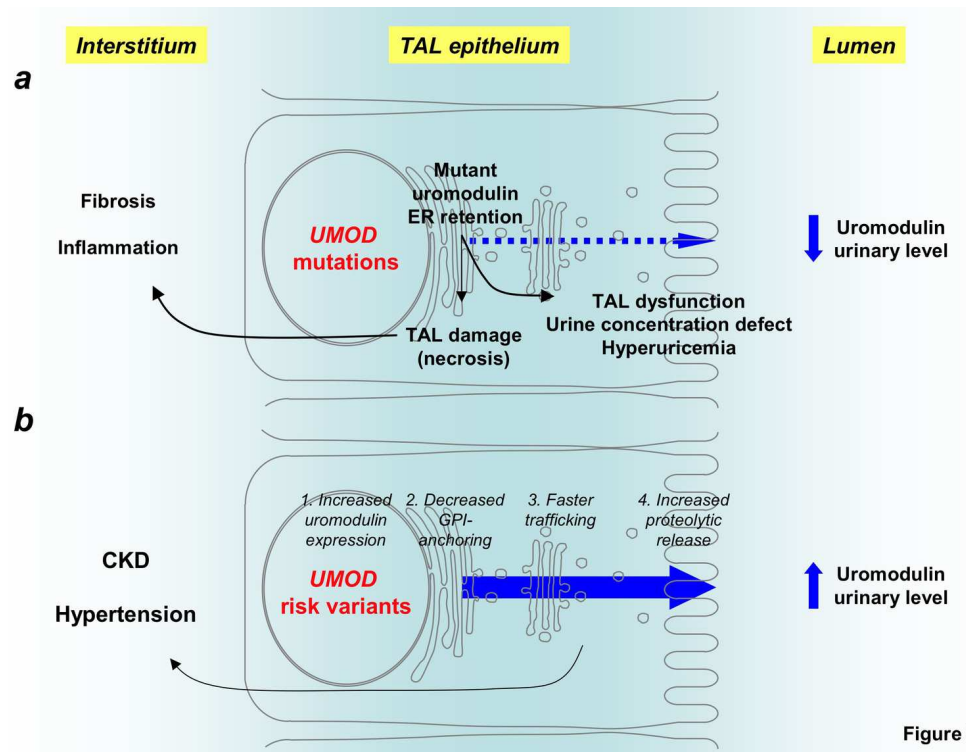


Figure 3

64x48mm (600 x 600 DPI)